## **WE CLAIM**

- 1. A composition comprising a pair of FRET hybridization probes hybridizing to a target nucleic acid sequence, each of said pair of FRET hybridization probe comprising
  - a nucleotide sequence entity, said nucleotide sequence entity being substantially complementary to a portion of the sequence of the target nucleic acid;
  - a fluorescent entity, said fluorescent entity being either a FRET donor entity or a FRET acceptor entity; and
  - a spacer entity, said spacer entity connecting the nucleotide sequence entity and the fluorescent entity;

wherein the FRET hybridization probes hybridize adjacently to each other on the target nucleic acid; and

wherein the spacer entities of the FRET hybridization probes are capable of forming non covalent interactions with each other.

- 2. A composition according to claim 1, wherein said non covalent interactions are nucleotide base pairing interactions.
- 3. A composition according to claim 2, wherein said nucleotide base pairing interactions are A/T base pairing interactions.
- 4. A composition according to claim 1, wherein said fluorescent entities on said pair of FRET hybridization probes are selected from the group consisting of fluorescein/Cy5, fluorescein/LC Red 640, fluorescein/LC Red 705, and fluorescein/JA286.
- 5. A composition according to claim 1, wherein said FRET acceptor entity is a Dabcyl or a Black Hole Quencher.
- 6. A composition according to claim 1, wherein at least one of the hybridization probes includes a nucleotide having a non-natural base.
- 7. A composition according to claim 6, wherein the non-natural base is selected from the group consisting of a 7-deazapurine, a diamino purine and a C-nucleotide.

- 8. A composition according to claim 1, wherein at least one of the hybridization probes includes a modified sugar-phosphate backbone.
- 9. A composition according to claim 8, wherein the modified sugar-phosphate backbone includes a 2-O methyl group or a phosphothioate.
- 10. A composition according to claim 1, wherein one of the hybridization probes is labeled at the 3' terminal end and the other of the hybridization probes is labeled at the 5' terminal end, such that upon hybridization of the probes to the target nucleic acid and excitation of the FRET donor entity, fluorescent resonance energy transfer to the FRET acceptor entity can occur.
- 11. A composition according to claim 1, wherein said non covalent interactions are hydrophobic interactions.
- 12. A composition according to claim 11, wherein said hydrophobic interactions are aryl/aryl, alkyl/alkyl or fluorinated hydrocarbons.
- 13. A composition according to claim 1, wherein said non covalent interactions are ionic interactions between a positively-charged group and a negatively-charged group.
- 14. A composition according to claim 1, wherein said spacer entity is branched.
- 15. A kit for use in performing a template dependent nucleic acid amplification reaction, comprising:
  - a pair of hybridization probes according to claim 1;

a container.

- at least one other component selected from the group consisting of nucleic acid amplification primers, a template dependent nucleic acid polymerase, deoxynucleoside triphosphates and a buffer suitable for use in a template dependent nucleic acid amplification reaction; and
- 16. A method for performing a template dependent nucleic acid amplification reaction, comprising:

- a) contacting a sample containing a target nucleic acid sequence with a pair of hybridization probes according to claim 1 in the presence of nucleic acid amplification primers, a template dependent nucleic acid polymerase, and deoxynucleoside triphosphates, in a buffer solution suitable for use in a template dependent nucleic acid amplification reaction under conditions suitable for amplification and hybridization of said hybridization probes to said target nucleic acid sequence;
- b) performing a template dependent nucleic acid amplification reaction;
- c) illuminating said sample with light at a frequency suitable for exciting the donor FRET moiety;
- d) detecting either an increase in emission of light from said acceptor FRET moiety or a decrease in emission of light from said donor FRET moiety; and
- e) repeating steps a-d a predetermined number of times.
- 17. The method of claim 16, wherein the template dependent nucleic acid amplification reaction is a polymerase chain reaction.
- 18. The method of claim 16, further comprising, after step e), changing the temperature of the sample and determining either an increase in emission of light from said acceptor FRET moiety or a decrease in emission of light from said donor FRET moiety as a function of temperature.
- 19. A method for performing a real-time template dependent nucleic acid amplification reaction, comprising:
  - a) contacting a sample containing a target nucleic acid sequence with a pair of hybridization probes according to claim 1 in the presence of nucleic acid amplification primers, a template dependent nucleic acid polymerase, and deoxynucleoside triphosphates, in a buffer solution suitable for use in a template dependent nucleic acid amplification reaction under conditions suitable for amplification and hybridization of said hybridization probes to said target nucleic acid sequence;
  - b) performing a template dependent nucleic acid amplification reaction;
  - c) repeating steps a-d a predetermined number of times; then
  - d) illuminating the sample with light at a frequency suitable for exciting the donor FRET moiety; then

- e) detecting either an increase in emission of light from said acceptor FRET moiety or a decrease in emission of light from said donor FRET moiety.
- 20. The method of claim 19, wherein the template dependent nucleic acid amplification reaction is a polymerase chain reaction.
- 21. The method of claim 19, further comprising, after step e), changing the temperature of the sample and determining either an increase in emission of light from said acceptor FRET moiety or a decrease in emission of light from said donor FRET moiety as a function of temperature.
- 22. A method for detecting a target nucleic acid sequence in a sample, comprising:
  - a) providing a pair of hybridization probes according to claim 1;
  - b) contacting the target nucleic acid with the pair of hybridization probes, under conditions suitable for hybridization of the hybridization probes to the target nucleic acid sequence; and
  - c) detecting the hybridization of the hybridization probes to the target nucleic acid sequence.
- 23. The method of claim 22, wherein the hybridization probes and the target nucleic acid are in solution.
- 24. The method of claim 22, wherein the target nucleic acid sequence is bound to a solid support.
- 25. The method of claim 22, wherein one of said hybridization probes is bound to a solid support.
- 26. The method of claim 22, wherein said step of detecting is performed by illuminating the sample with light at a frequency suitable for exciting the donor FRET moiety; then detecting either an increase in emission of light from said acceptor FRET moiety or a decrease in emission of light from said donor FRET moiety.
- 27. A reaction mixture for use in a dependent nucleic acid amplification reaction, comprising, in a solution:

a pair of hybridization probes according to claim 1; and
at least one other component selected from the group consisting of nucleic acid
amplification primers, a template dependent nucleic acid polymerase,
deoxynucleoside triphosphates and a buffer suitable for use in a template dependent
nucleic acid amplification reaction.